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(71) Applicant *(for all designated States except US)*: INDOOR BIOTECHNOLOGIES LIMITED [GB/GB]; 123 Deansgate, Manchester M3 2BU (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: CHAPMAN, Martin, Dudley [GB/US]; 1717 King Mountain Road, Charlottesville, VA 22901 (US).

(74) Agents: O'BRIEN, Caroline, J. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).

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(54) Title: ALLERGEN DETECTION ASSAY

(57) Abstract

The invention provides a method for testing for the presence of a particular allergen which comprises (a) obtaining a substantially unpurified sample suspected of containing an allergen; (b) if a non-liquid sample mixing it with a liquid diluent and if a liquid sample optionally diluting it with liquid diluent to produce a liquid sample being a solution and/or suspension of the sample; (c) contacting the liquid sample or a portion thereof with a labelled first binding reagent for a said allergen which reagent is freely movable through a porous support of an assay device; (d) allowing the first binding reagent whether free or complexed to allergen to move along a said porous support to meet a second binding reagent for a said allergen immobilised at a detection zone in the support, wherein the first and second binding reagents are with respect to each other, either competitive or non-competitive for said allergen and a said binding reagent is specific for an epitope characteristic of said allergen; and (e) examining for labelled binding reagent at the detection zone, the presence of labelled binding reagent at the detection zone being indicative of the absence/presence of allergen in the sample, depending on whether the first and second binding reagents are with respect to each other, competitive or non-competitive for said allergen.

ALLERGEN DETECTION ASSAY

The present invention relates to an assay for detecting the presence of environmental allergens in eg foods, air, dust such as house dust, and in particular to a one-step rapid assay of the "dipstick" type.

BACKGROUND OF INVENTION

Allergens present in the environment (eg indoor allergens such as occur in the home) are a major risk factor in the onset of allergic diseases or in exacerbating the symptoms of such disease. (T.A.E.Platts-Mills, Annals of Allergy, (1994), 72, 4, 381-384). Particular examples of such diseases are rhinitis, asthma, hayfever or eczema.

Patients may suffer an allergic response if exposed to allergens such as plant or animal allergens present in eg food, dust etc. Examples of the most common domestic allergens which are implicated in allergic diseases include, for example, those derived from house dust mites or insects such as cockroaches and flies, domestic pets such as cats and dogs, and plant allergens such as pollen, as well as fungal allergens. Other allergens may be presented by foodstuffs such as nuts, or industrial substances such as latex.

Sensitivity to allergens is an increasing problem. In some cases, it appears that patients have a genetic predisposition to develop allergies following exposure to allergens in their environment. Some patients become sensitised to particular allergens which are present at high levels in their environment. For instance, in regions of the world where house dust mites are prevalent, it is found that a large number of patients suffering from asthma are allergic to house dust mite allergen. However, in other areas, for example where the

Review of Respiratory Disease 1981: 124:630-636, or T.A.E. Platts-Mills et al, Allergy and Clinical Immunology 1992: 89: No 5,pp1046-1060. The devices used include moulded plastic attachments for fitting on the wand of a vacuum cleaner and which contain a membrane of paper filter, inside upon which dust is collected (eg as available from ALK Laboratories, Horsholm, Denmark). A further device, available from Allergy Control Products, Ridgefield, Connecticut, USA comprises a fiber bag-like receptacle with a plastic rim portion, which fits on the end of the vacuum cleaner so that the bag fits inside the pipe. When the vacuum cleaner is used, dust collects in the bag and can then be extracted.

Collected dust is then sent to laboratories where allergens are extracted and they are tested for using the microplate based enzyme-linked immunoabsorbent assay (ELISA) tests. Typically, in these tests, antibodies are absorbed onto a plastic surface, allergen extract is added, and bound allergen detected using a labelled second antibody. The second antibody is detected using a ligand coupled to an enzyme which can be visualised by addition of a chromogen. However this technology is difficult to adapt for home use and cannot be used by patients themselves. A review of immunoassays for indoor allergens can be found in Chapter 11 of "Bioaerosols" Ed.Harriet A. Burge, (1995), CRC Press Inc..

Another type of test available specifically for house dust mites is the so-called "guanine test" (Bischoff E et al., Allergologie 1984: 11: 446-449, JEM Van Bronswijk et al., J. Medical Entomology 1989: 26: 55-59). In this test, house dust samples are tested chemically for the presence of guanine which is indicative of the presence of house dust mites using a dipstick type test. The results obtainable using this test are not altogether satisfactory (M. D. Chapman, Allergy, 1993, 48: 301-302)

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a dipstick for use in the assay: (a) shows the device prior to use and (b) shows the device after use with a typical positive result;

Figures 2, 3 and 4 show a series of developed dipsticks used in assays for Fel d 1 in house dust obtained from homes in UK, USA and Brazil;

Figures 5, 6, 7 and 8 show a series of developed dipsticks used in assays for mite Group 2 allergens Der p 2 and Der f 2 in house dust obtained from different locations in homes in Brazil.

The present invention provides an assay suitably carried out as a one-step "dipstick" type assay. Thus the invention provides an assay for detecting the presence of allergens in a sample (such as dust), which comprises use of a dipstick and reagents which are capable of specifically binding to and detecting a particular allergen which may be present in a sample (such as dust).

DESCRIPTION OF THE INVENTION

According to the present invention there is provided a method for testing for the presence of a particular allergen which comprises: (a) obtaining a substantially unpurified sample suspected of containing an allergen; (b) if a non-liquid sample mixing it or a portion thereof with a liquid diluent and if a liquid sample optionally diluting it or a portion thereof with liquid diluent to produce a liquid sample being a solution and/or suspension; (c) contacting the liquid sample or a portion thereof with a labelled first binding reagent for a said allergen, the reagent being freely movable through a porous support of an assay device; (d) allowing the first binding reagent whether free or complexed to allergen to move along a said porous support to meet a second binding

The first and second binding reagents may both comprise substances with a monoclonal antibody binding domain with specificity for said allergen.

The first and second binding reagents may both comprise either a monoclonal antibody or a monoclonal antibody fragment.

The first and second binding reagents may both comprise either a monoclonal antibody or a monoclonal antibody fragment, which antibody or fragment has an antibody binding domain with specificity a said epitope characteristic of said allergen.

The allergen may be one of cat, cockroach, aspergillus or dust mite.

The method may be for testing for the presence of mite Group 1 allergens, or mite Group 2 allergens or both mite Group 1/Group 2 allergens.

The porous support may comprise a nitrocellulose membrane.

The porous support may be accommodated within a housing.

The labelled first binding reagent may comprise a gold label.

The absence/presence of allergen in the sample may be determinable within 20 minutes of contacting the liquid sample with said labelled first binding reagent. The absence/presence of allergen in the sample may be determinable within 15 minutes. The absence/presence of allergen in the sample may be determinable within 10 minutes. The absence/presence of allergen in the sample may be determinable within 5 minutes.

moveable through said support, and (c), a second binding reagent which specifically binds either said allergen in a manner complementary to that of the first binding reagent or which competes with said allergen for binding to said first binding reagent said second binding reagent being immobilised at a detection site on the support.

Also provided are assay systems for testing for the presence of a particular allergen which may be found in a substantially unpurified sample suspected of containing an allergen which comprises (a) a porous support, (b) a labelled first binding reagent for a said allergen, the reagent being freely movable through a porous support of an assay device, (c) a second binding reagent for said allergen which second reagent is immobilised at a detection zone in the support and wherein the first and second binding reagents are with respect to each other, either competitive or non-competitive for said allergen and a said binding reagent is specific for an epitope characteristic of said allergen.

The porous support may bear a solution or suspension of the substantially unpurified sample suspected of containing an allergen.

The labelled first binding reagent may be in a vessel external to the support.

The labelled first binding reagent may be in the porous support.

The second binding reagent may specifically bind said allergen in a manner complementary to that of the first binding reagent.

The first and second binding reagents may be with respect to each other non-competitive for said allergen.

The porous support may be accommodated within a housing.

The labelled first binding reagent may comprise a gold label.

The absence/presence of allergen in the sample may be determinable within 20 minutes of contacting the liquid sample with said labelled first binding reagent. The absence/presence of allergen in the sample may be determinable within 15 minutes. The absence/presence of allergen in the sample may be determinable within 10 minutes. The absence/presence of allergen in the sample may be determinable within 5 minutes.

Also provided are assay systems as above for testing for the presence of a plurality of particular allergens which may be found in the sample.

Such an assay system may utilise a plurality of porous supports, each support being for testing for the presence of one particular allergen of the plurality of particular allergens.

Alternatively, such an assay system may utilise a single porous support for testing for the presence of more than one particular allergen of the plurality of particular allergens.

An assay system for testing for the presence of a plurality of particular allergens which may be found in the sample may comprise a plurality of pairs of first and second binding reagents, each pair being for testing for the presence of one particular allergen of the plurality of particular allergens.

A common first or second binding reagent may be employed for testing for the presence of more than one particular

depending on the precise nature of the label, either directly, or by use of some signal developing reagent.

Alternatively, if the second binding reagent competes with said allergen for binding to said first binding reagent, the complex will not be fixed in the detection zone and the absence or relative absence of a signal from said label indicates that the sample contains allergen (a competitive assay).

Preferably, for home use, the assay device is arranged as a sandwich type assay.

In a preferred embodiment, the assay is sufficiently sensitive to allow detection of allergens present in a extract formed by mixing a sample such as dust with a diluent such as water or buffered saline. The level of sensitivity is suitably such that it will detect allergens which are present in concentrations which are high enough to be clinically significant in that they are likely to have an affect on allergic patients. This will generally mean that the assay should detect the presence of allergens at levels of less than 50 ng, suitably less than 5 ng and sometimes less than 300 pg although this will depend to a certain extent on the nature of the samples, the allergen being detected, extraction volumes and conditions. Assays exemplified herein show sensitivities of around 100 pg.

Suitably at least one of the first and second binding reagents comprises a substance with an antibody binding domain eg antibodies or binding fragments thereof which retain specificity for said allergen. Antibodies may be polyclonal. Preferably at least one of the first and second binding reagents comprises a substance with an antibody binding domain of a monoclonal antibody. Antibodies to specific allergen may be generated using

Cockroach (*Blattella germanica*) Bla g 1	mAb 10A6 Rabbit polyclonal anti-CR antiserum
Cockroach (*Blattella germanica*) Bla g 2	mAb 7C11 Biotinylated mAb 8F4
Aspergillus (*A. fumigatus*) Asp f 1	mAb 4A6 Rabbit polyclonal anti-Asp f 1

The above-mentioned antibodies are available from Indoor Biotechnologies Ltd of 123 Deansgate, Manchester, M3 2BU, UK.

Allergen assays for detecting either mite Group 1 allergens or mite Group 2 allergens or both mite Group 1 and Group 2 allergens may be provided. Group 2 allergens are produced by at least two species of house dust mite and therefore detection indicates the presence of either of these species. There may be advantages to detecting both allergens. Group 2 allergens are less easily destroyed than Group 1 allergens and therefore assessment of precisely which allergens were present would provide a better indication as to which chemical avoidance treatments are required.

Suitably the porous support material is a nitrocellulose membrane, such as those obtainable from Advanced Microdevices (Pvt) Ltd, 20-21 Industrial Area, Amblata Cantt, 13001 India, which may be held or cast onto a rigid backing support for example of a plastics material. Suitably the pore size of the membrane is between 5-15μ, suitably about 8μ and the membrane thickness is in the range of 75-125μ, suitably about 95μ. It may be cast onto a backing material such as mylar, and supported in a plastics support, for instance a HIPS plastic support. The backing material will suitably be of a thickness with is similar to or slightly greater than that of the

which applies biochemical reagents. A preferred configuration is to apply the second binding reagent as a thin line in the detection zone. This ensures that any labelled first reagent which binds in this area is concentrated as far as possible to provide a clear signal.

Preferably the assay is arranged so that a visible signal develops within a relatively short time period for example of less than 20 minutes, suitably within 5 to 15 minutes.

By providing a rapid assay which can specifically detect the presence of particular allergens in eg the home environment, patients suffering from allergic disease will be able to able to monitor their own exposure to those allergens and this should encourage compliance with avoidance procedures. Additionally, families with a genetic predisposition to asthma can monitor their environment and take action to avoid the onset of asthma in children of the family.

Furthermore, the assay is very specific for particular allergens such as are found in house dust and also highly sensitive. Detection limits of 100pg for the Der p 1 allergen, 50 to 100pg for the mite Group 2 allergens, 100pg for the Fel d 1 and 200pg for Bla g 2 allergen, are representative of levels which may be achieved. This is important when dealing with dust extracts where the concentration of allergens available to move through the porous support is very low.

The tests may be sold individually or in combination. A range of rapid test allergen assay devices for different allergens may be sold together as a kit to allow a complete allergen analysis of a particular environment to be carried out. The kits may comprise a series

Detector antibody (4C1 dust mite) at a concentration of 5.2μg/ml was added to each member in a dilution series of solutions made from *Dermatophagoides pteronussinius* extract (10,000 AU/ml) containing 25μg/ml Der p 1 allergen and a control solution which contained no allergen extract.

An end (3) of a dipstick was immersed in each of the thus prepared solutions and the solution allowed to travel along the dipstick to the detection zone. Within a period of 5 to 10 minutes, a red line had appeared in the detection zone of all the dipsticks except those which had been treated with control solution.

Example 2

The process of Example 1 was repeated but instead of the Der p 1 allergen, extracts containing the following allergens were tested using the given combination of capture and detection antibody:

Allergen Extract	Capture Antibody	Detection Antibody
Der f 1	6A8	Gold labelled 4C1 (5.2μg/ml)
Der p 2	1D8	Gold labelled 7A1 (6.0μg/ml)
Fel d 1	6F9	Gold labelled 3E4 (5.4μg/ml)

Similar results to those found in Example 1 were detected.

Example B

Example 2 was repeated, but instead of allergen extract, samples of house dust extract were used. House dust was collected from a number of different houses using a vacuum cleaning device with a dust collector attached.

The results are shown in Fig. 2. Each of the dipsticks in the Fig.2 control curve series is for a different concentration of allergen starting at 80 ng/ml and going down to 1ng/ml as set out above. The assay sensitivity is approximately 2.5 ng/ml, equivalent to about 100pg per test (as determined by a quantitative plate-type enzyme immunoassay (EIA) to measure the allergen Fel d 1 in the dust samples).

Example 5

Assay devices as described in Example 4 were prepared and used. In this example the dust was collected from the air filters of vacuum cleaners used in US homes. The results are shown in Fig. 3. In most cases the dust sample contained Fel d 1 detectable by the dipstick assay device. The dust samples were separately assayed by EIA as stated in Example 4. Where the dipsticks in Fig. 3 are marked ▼, EIA results gave a dust sample Fel d 1 concentration of less than 1 mU/ml. Where the dipsticks in Fig. 3 are marked •, EIA results gave a dust sample Fel d 1 concentration of greater than 100 mU/ml.

Example 6

Assay devices as described in Example 4 were prepared and used. Bedding dust was collected from a number of different homes in São Paulo, Brazil using a vacuum cleaning device with a dust collector attached. The dust was sieved in order to remove large debris and a 100mg sample of the remainder was shaken in the presence of 2ml of buffered saline in order to provide dust samples for assay. Some of the homes had a cat in residence.

The results are shown in Fig. 4. In a number of cases, the bedding dust sample contained Fel d 1 detectable by the dipstick assay device. The dust samples were separately assayed by EIA as stated in Example 4. Where the dipsticks in Fig. 4 are marked ▼, EIA results gave a

250,125,63,32,16, 8,4,2 and 1 ng/ml Der f 2 allergen and a control solution which contained no allergen. These solutions comprise the Der f 2 control samples.

An end (3) of the dipstick carrying the 1D8 capture antibody which is directed against both Der p 2 and Der f 2 allergens, was immersed in each of the samples (dust: kitchen; bedroom floor and bedding; Der p2 control samples; and Der f 2 control samples).

The results are shown in Figs 5, 6, 7 and 8. Fig. 5 shows that the dipstick device has a sensitivity of 1-2 ng/ml, equivalent to about 50 to 100pg per test. The dipstick device was also able to detect the mite Group 2 allergens (Der p 2 and Der f 2) allergen in house dust samples obtained from the kitchen floors, bedroom floors and bedding.

The dust samples were separately assayed by EIA as stated in Example 4. Where the dipsticks in Fig. 6, 7 and 8 are marked ▼, EIA results gave dust sample mite Group 2 (Der p 2 and Der f 2 allergens) concentrations of less than 1μg/g. Even at this low level of allergen concentration the dipstick was able to detect the presence of the allergens in samples from kitchen floors, bedroom floors and bedding. Where the dipsticks in Fig. 6 are marked •, EIA results gave a dust sample mite Group 2 allergens(Der p 2 and Der f 2) concentrations of greater than 10μg/g.

contacting the liquid sample or a portion thereof with said labelled first binding reagent occurs in the porous support.

3. A method according to claim 1 wherein the step of contacting the liquid sample or a portion thereof with said labelled first binding reagent occurs in a vessel external to the support.

4. A method according to any one of claims 1 to 3 wherein said first and second binding reagents are with respect to each other non-competitive for said allergen.

5. A method according to any one of claims 1 to 3 wherein said first and second binding reagents are with respect to each other competitive for said allergen.

6. A method according to any one of claims 1 to 5 wherein a said binding reagent comprises a substance with an antibody binding domain specific for said allergen.

7. A method according to claim 6 wherein the antibody binding domain derives from a monoclonal antibody.

8. A method according to any one of claims 1 to 7 wherein a said binding reagent comprises a monoclonal antibody or a fragment thereof.

9. A method according to any one of claims 1 to 8 wherein said first and second binding reagents both comprise substances with a monoclonal antibody binding domain specific for said allergen.

10. A method according to claim 9 wherein said first and second binding reagents both comprise either a monoclonal antibody or a monoclonal antibody fragment.

determinable within 10 minutes.

20. A method according to claim 17 wherein the absence/presence of allergen in the sample is determinable within 5 minutes.

21. A method according to any one of claims 1 to 20 for testing for the presence of a plurality of particular allergens which may be found in the sample.

22. A method according to claim 21 which utilises a plurality of porous supports, each support being for testing for the presence of one particular allergen of the plurality of particular allergens.

23. A method according to claim 21 which utilises a single porous support for testing for the presence of more than one particular allergen of the plurality of particular allergens.

24. A method according to any one of claims 21 to 23 which comprises a plurality of pairs of first and second binding reagents, each pair being for testing for the presence of one particular allergen of the plurality of particular allergens.

25. A method according to claim 24 wherein a common first or second binding reagent is employed for testing for the presence of more than one particular allergen of the plurality of particular allergens.

26. A method according to any one of the preceding claims wherein the sample is selected from dust, water, air, a foodstuff or a drink.

27. A method according to claim 26 wherein the sample is a dust sample.

35. An assay system according to any one of claims 30 to 34 wherein a said binding reagent comprises a substance with an antibody binding domain specific for said allergen.

36. An assay system according to claim 35 wherein the antibody binding domain derives from a monoclonal antibody.

37. An assay system according to any one of claims 30 to 36 wherein a said binding reagent comprises a monoclonal antibody or a fragment thereof.

38. An assay system according to any one of claims 30 to 37 wherein said first and second binding reagents both comprise substances with a monoclonal antibody binding domain specific for said allergen.

39. An assay system according to claim 38 wherein said first and second binding reagents both comprise either a monoclonal antibody or a monoclonal antibody fragment.

40. An assay system according to claim 39 wherein said first and second binding reagents both comprise either a monoclonal antibody or a monoclonal antibody fragment, which antibody or antibody fragment has an antibody binding domain specific for a said epitope characteristic of said allergen.

41. An assay system according to any one of claims 30 to 40 wherein the allergen is one of cat, cockroach, aspergillus or dust mite.

42. An assay system according to claim 41 for testing for the presence of mite Group 1 allergens, or mite Group 2 allergens or both mite Group 1/Group 2 allergens.

52. An assay system according to claim 50 which utilises a single porous support for testing for the presence of more than one particular allergen of the plurality of particular allergens.

53. An assay system according to any one of claims 50 to 52 which comprises a plurality of pairs of first and second binding reagents, each pair being for testing for the presence of one particular allergen of the plurality of particular allergens.

54. An assay system according to claim 53 wherein a common first or second binding reagent is employed for testing for the presence of more than one particular allergen of the plurality of particular allergens.

55. An assay system according to any one of claims 30 to 54 wherein the sample is selected from dust, water, air, a foodstuff or a drink.

56. An assay according to claim 55 wherein the sample is a dust sample.

57. An assay according to claim 55 or claim 56 wherein the sample is a house dust sample.

58. An assay system as described herein with reference to one or more of examples 1 to 7.

Fig. 2

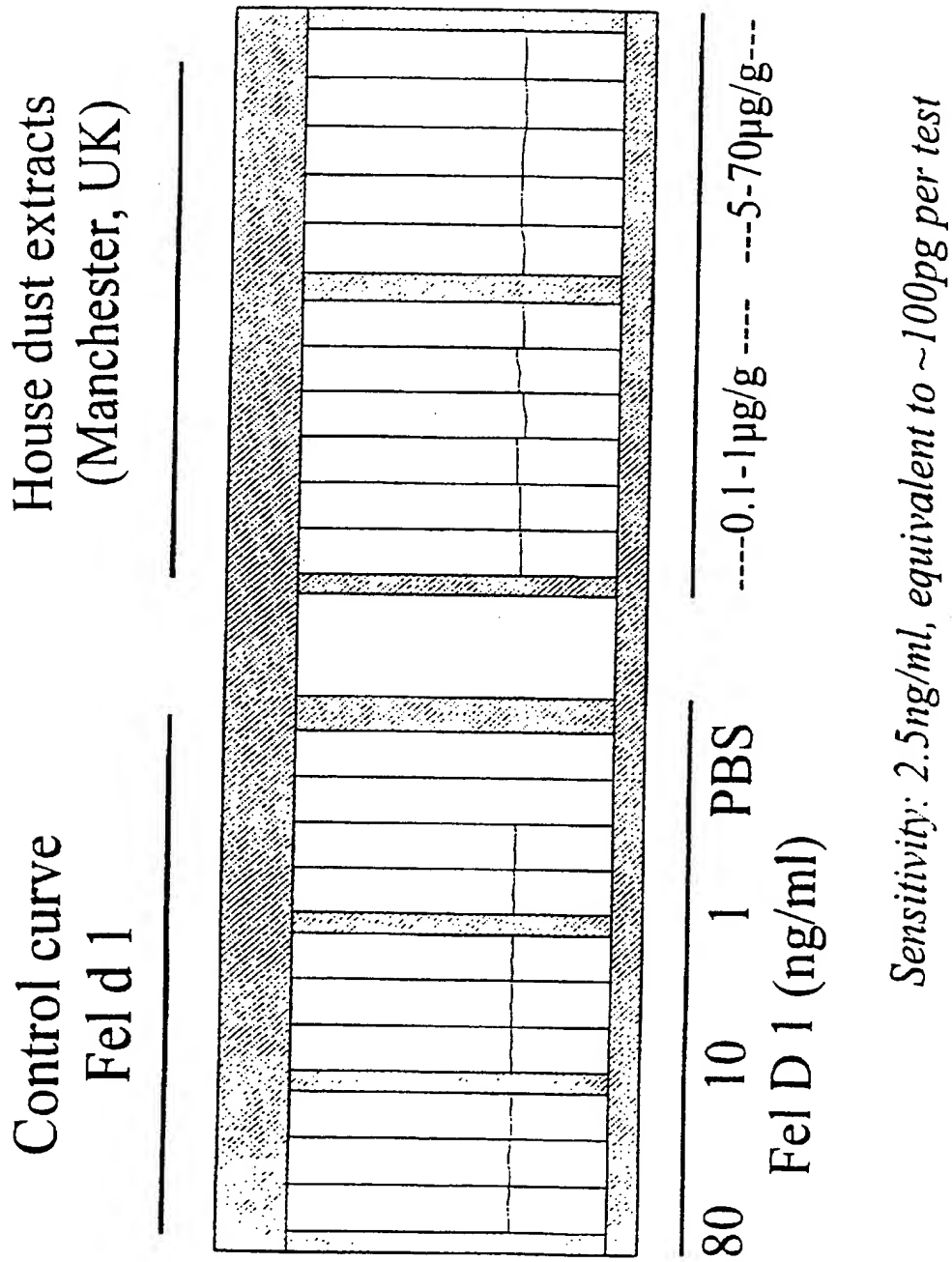
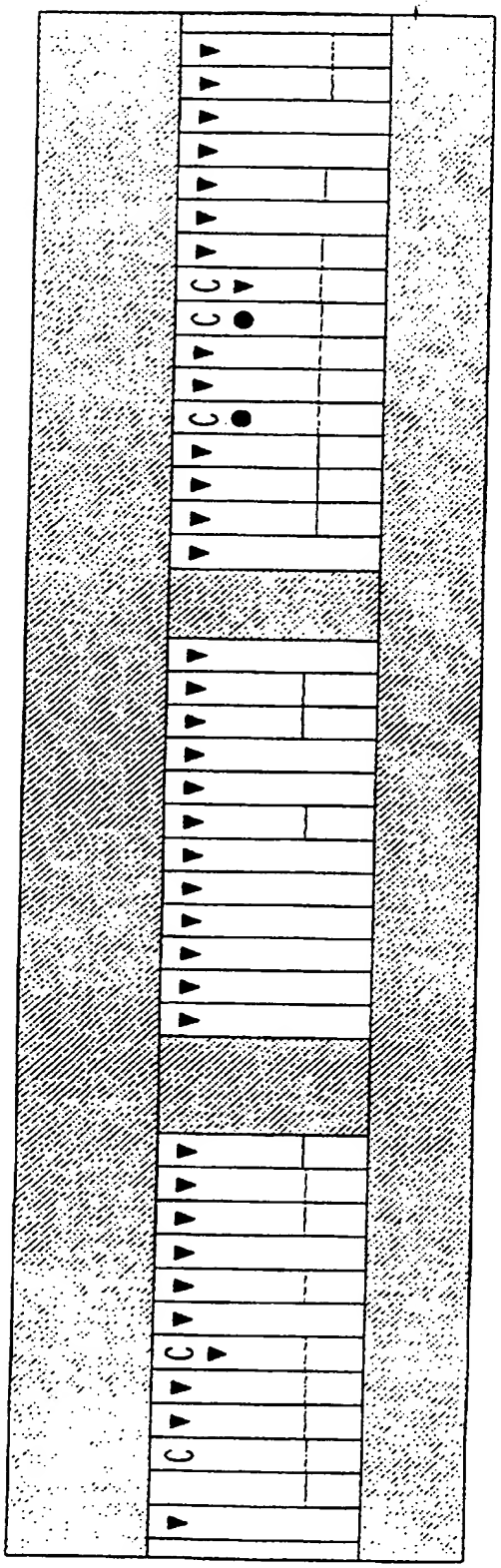


Fig. 4

Fel d 1 in House Dust Extracts from São Paulo, Brazil (n=40)



C = homes with cat or cat in yard; ▼ =<1µg/g Fel d 1; ● =≥8µg/g

Fig. 6

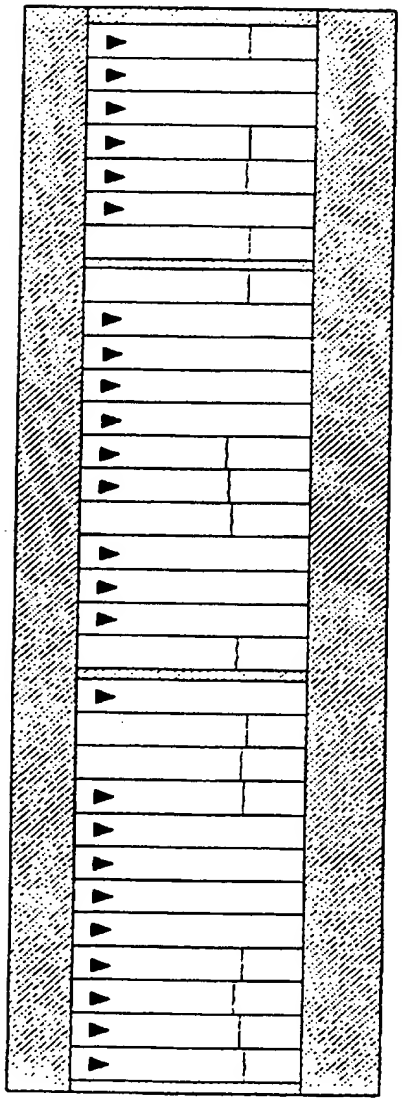
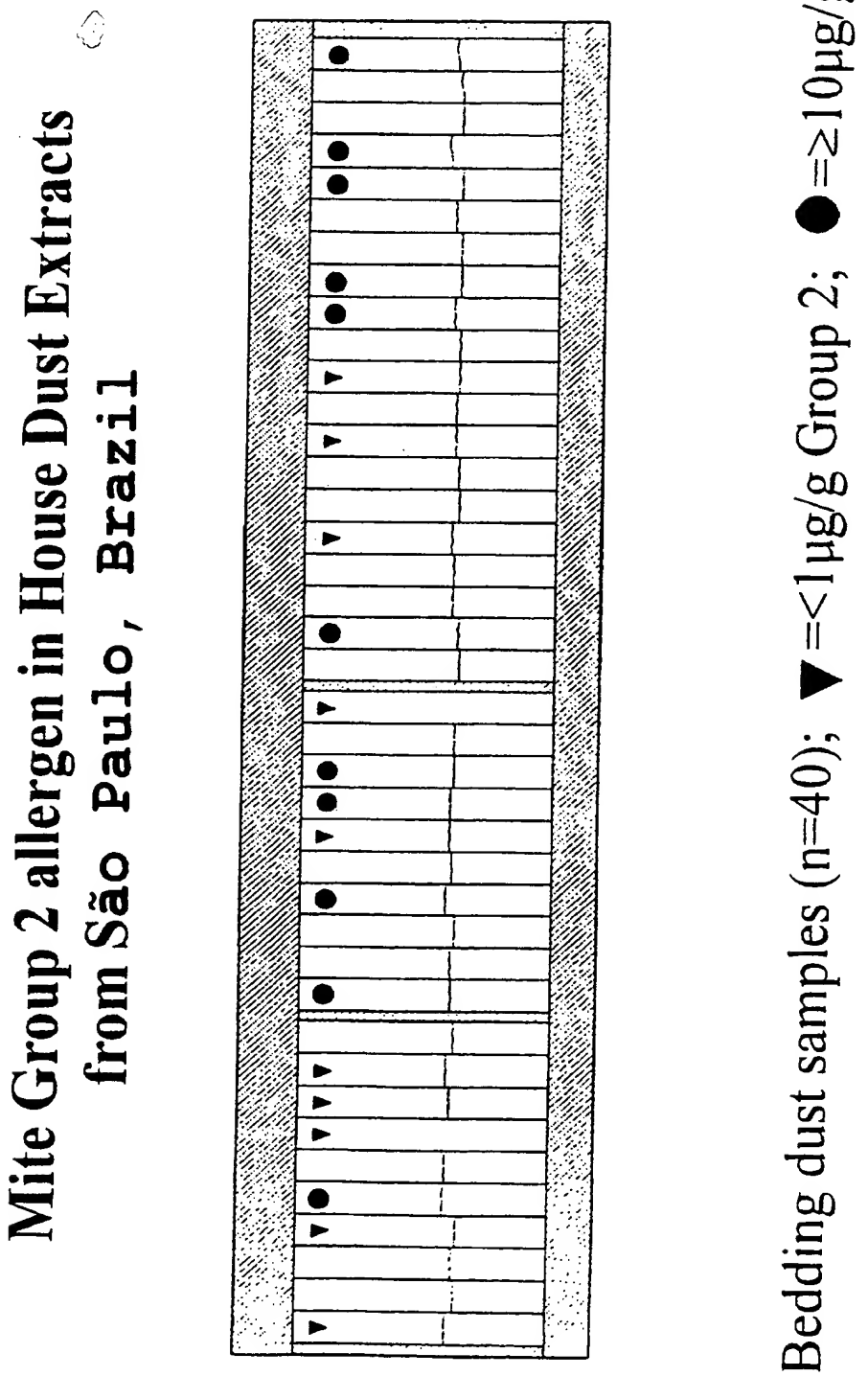


Fig. 8


Mite Group 2 allergen in House Dust Extracts
from São Paulo, Brazil
Bedding dust samples (n=40); ▼=<1µg/g Group 2; ●=≥10µg/g

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00223

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 498 124 A (ASAHI BREWERIES, LTD.) 12 August 1992 cited in the application	

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